

CLAIMS

1. A method of screening a novel protein or partial sequence protein candidate for blocking having a blocking ability based on amino acid sequence data, the method of screening the protein or  
5 partial sequence protein, which meets the following conditions:  
A) the amino acid sequence of the protein is divided into two, and an absolute value of a difference between hydrophilic/hydrophobic rates in divided two portions is 0.1 or more, calculated using the following formula from content rates  
10 of hydrophilic amino acids (D, E, K, H, R, Y) and hydrophobic amino acids (G, A, V, L, I, M, F, W, P);  
[Hydrophilic/hydrophobic rate] = [Content rate of hydrophilic amino acids]/[Content rate of hydrophobic amino acids];  
B) the hydrophilic/hydrophobic rate in a hydrophilic portion (a  
15 higher value of hydrophilic/hydrophobic rate) is 0.5 or more; and  
C) the protein is composed of more than 100 amino acid residues.
  
2. A novel protein or a novel partial sequence protein for blocking having a blocking ability, screened by the method of  
20 claim 1, the protein or the partial sequence protein which can be obtained by the following analysis step D and meets the following condition E;  
D) (1) a step of adding a candidate protein (0.5 to 1 mg/mL, diluted with 20 mM Tris-HCl, pH 7.0) which meets the conditions  
25 of claim 1 and bovine serum albumin (fraction V) prepared by the same way to respective wells of a polystyrene immunotiter plate, blocking at 2 to 10°C for 4 to 5 hours and removing solutions;  
(2) a step of adding normal human serum diluted 25 to 100 times with PBS(-), leaving stand at 37°C for one hours, and subsequently  
30 washing the plate with PBS(-) (0.05% Tween 20); and  
(3) a step of comparing IgG amounts non-specifically absorbed to the plate using an enzyme-labeled anti-human IgG antibody by a colorimetric method using a chromogenic substrate; and  
E) the absorbance for the candidate protein is 2.5 times or less  
35 than the developed color intensity for bovine serum albumin.

3. A novel protein or a novel partial sequence protein for blocking having a blocking ability, screened by the method of claim 1, the protein or the partial sequence protein which can be  
5 obtained by the following analysis step F and meets the following condition G;

F) (1) a step of dissolving horseradish peroxidase for labeling at 0.05 mg/mL in a candidate protein solution (0.5 to 1 mg/mL, diluted with PBS(-)) and a bovine serum albumin (fraction V)

10 solution prepared by the same way;

(2) a step of dispensing the above diluted solution to a polystyrene 96-well microplate;

(3) a step of leaving stand at 25°C for one hour, removing the solution and washing with PBS(-) containing 0.02% Tween 20;

15 (4) a step of adding a tetramethylbenzidine solution, incubating at 37°C and subsequently adding 1N sulfuric acid to stop a reaction and develop a color; and

(5) a step of measuring the absorbance by a microplate reader; and

20 G) the absorbance for the candidate protein is 2.5 times or less than the developed color intensity for bovine serum albumin.

4. A novel protein or a novel partial sequence protein for blocking having a blocking ability, screened by the method of  
25 claim 1, the protein or the partial sequence protein which can be obtained by the following analysis step H and meets the following condition I;

H) (1) a step of adding a candidate protein (0.5 to 1 mg/mL, diluted with 20 mM Tris-HCl, pH 7.0) which meets the conditions

30 of claim 1 and bovine serum albumin (fraction V) prepared by the same way to respective wells of a polystyrene immunotiter plate, blocking at 2 to 10°C for 4 to 5 hours and removing solutions;

(2) a step of adding a peroxidase solution prepared at 0.05 mg/mL, leaving stand at 37°C for one hour and subsequently washing the

35 plate with PBS(-) (0.05% Tween 20);

- (3) a step of leaving stand at 25°C for one hour, subsequently removing the solution and washing with PBS(-) containing 0.02% Tween 20; and
- (4) a step of adding a tetramethylbenzidine solution, incubating  
5 at 37°C and subsequently adding 1N sulfuric acid to stop a reaction and develop a color; and
- (5) a step of measuring the absorbance by a microplate reader; and
- I) the absorbance for the candidate protein is 2.5 times or less  
10 than the developed color intensity for bovine serum albumin.

5. A novel protein achieving an improved blocking efficiency by modifying an amino acid sequence of a protein or a partial sequence protein which meets the conditions A, B and C according  
15 to claim 1.

6. The protein achieving the improved blocking efficiency according to claim 5 characterized in that the amino acid sequence is modified by amino acid substitution, deletion and  
20 insertion.

7. The protein achieving the improved blocking efficiency according to claim 5 characterized by being derived from a prokaryotic organism or an eukaryotic organism.  
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8. The protein achieving the improved blocking efficiency characterized by being derived from an "HSP70 family protein".

9. The protein achieving the improved blocking efficiency  
30 according to claim 8 characterized by being derived from a DnaK protein.

10. The protein achieving the improved blocking efficiency according to claim 8 characterized by being a protein obtained by  
35 deleting a part of an amino acid sequence of the DnaK protein.

11. The protein achieving the improved blocking efficiency according to according to 8, which is a protein obtained by deleting a part of an amino acid sequence of the DnaK protein,  
5 characterized in that an amino acid sequence from an N terminus to at least position 387 and at most position 472 has been deleted.

12. The protein achieving the improved blocking efficiency according to according to 8, which is a protein obtained by deleting a part of an amino acid sequence of the DnaK protein,  
10 characterized in that an amino acid sequence from an N terminus to at least position 387 and at most position 418 has been deleted.

13. The protein according to claim 8 composed of an amino acid sequence of positions 419 to 607 in the DnaK protein.  
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14. The protein achieving the improved blocking efficiency according to claim 8 characterized in that a part of hydrophilic amino acids is substituted with hydrophobic amino acids in the DnaK protein wherein an ATPase domain or a part thereof has been deleted.  
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15. The protein achieving the improved blocking efficiency according to claim 8 which is a protein wherein a part of an amino acid sequence is deleted in the DnaK protein wherein an ATPase domain or a part thereof has been deleted, wherein aspartic acid at positions 479 and 481 in the amino acid sequence  
25 is substituted with valine.  
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16. The protein achieving the improved blocking efficiency according to claim 8 composed of an amino acid sequence of positions 384 to 607 in the DnaK protein, wherein aspartic acid  
35 at positions 479 and 481 in the amino acid sequence has been

substituted with valine.

17. A protein for blocking having one or more hydrophilic domains and one or more hydrophobic domains, wherein the  
5 hydrophobic domain can be absorbed to a material surface and the hydrophilic domain can cover the hydrophobic domain absorbed to the material surface.
18. A modified protein characterized in that a blocking speed  
10 is further enhanced than that of BSA.
19. The modified protein according to claim 18 characterized in that a blocking ability in less than 10 minutes is more excellent than that of BSA under a condition where protein amounts are  
15 adjusted so as to exhibit a blocking efficiency equivalent to that of BSA in blocking for 3 hours.
20. The protein according any of claims 2 to 19 characterized by having a tag sequence.  
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21. The protein according to claim 20 characterized in that the tag sequence is selected from a histidine tag, a maltose binding protein (MBP) tag, a glutathione S-transferase (GST) tag, a Flag tag, a Myc tag, and a tandem affinity purification tag.  
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22. The protein according to any of claims 2 to 19 characterized in that an optional amino acid sequence is added.
23. A method of producing a protein characterized by producing  
30 the protein according to any of claims 2 to 22 using a prokaryotic organism.
24. A method of producing a protein characterized by producing  
35 the protein according to any of claims 2 to 22 using *Escherichia coli*.

25. A method of producing a protein characterized by producing the protein according to any of claims 2 to 22 using a cell-free protein synthesis method.

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26. A method of purifying the protein according to any of claims 2 to 22, characterized by passing through a heating step.

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27. A method of using the protein according to any of claims 2 to 22 for blocking, stabilization, size enlargement, protein folding promotion, protein refolding promotion, coating and medical use.

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28. A blocking reagent, a stabilizing agent, an excipient, a protein folding accelerator, a protein refolding accelerator, a coating agent for cell attachment or a coating agent for medical use, which contains the protein according to any of claims 2 to 22.

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